Synopsis

Enzymes are the molecular architects of biological systems, orchestrating a myriad of chemical reactions with unparalleled precision. The catalytic prowess of enzymes stems from their ability to facilitate reactions under mild conditions, ensuring optimal substrate conversion rates while minimizing energy expenditure. Traditionally, enzyme-catalyzed reactions were thought to occur in an unorganized environment, where substrates diffuse freely in solution to bind to the active site of the enzyme. However, emerging evidence has highlighted an alternative mode of enzyme-substrate interaction known as substrate channeling (Wheeldon et al. 2016).

In intricate metabolic pathways, the phenomenon of substrate channeling has emerged as a remarkable mechanism that enhances reaction efficiency by allowing intermediates to be transferred directly between active sites within multi-enzyme complexes. This process, which prevents the diffusion of labile intermediates into the bulk solution, has far-reaching implications for cellular metabolism, regulation, and biotechnological applications. In this comprehensive write-up, we delve into the concept of substrate channeling, elucidate its molecular mechanisms, explore experimental evidence supporting its existence, and discuss its profound impact on cellular function and biotechnology (Zhang 2011).

Furthermore, substrate channeling has been exploited in the construction of artificial metabolic pathways within cells. Synthetic biology approaches leverage the principles of substrate channeling to engineer pathways that mimic natural metabolic networks. This enables the creation of cells with enhanced biosynthetic capabilities, paving the way for the production of valuable compounds such as biofuels, pharmaceuticals, and specialty chemicals (Hand and Hardewig 2003).

Throughout human history, medicinal plants have been an integral part of healthcare systems across cultures, offering a plethora of natural remedies for various ailments. These plants, often gifted by nature with potent compounds and therapeutic properties, have played a significant role in alleviating illnesses and improving overall well-being. The profound connection between

humans and medicinal plants dates back millennia, and modern scientific research continues to uncover their remarkable potential in the realm of medicine (Fabricant and Farnsworth 2001).

Modern research techniques have unveiled the intricate biochemistry behind the healing properties of these plants. Compounds such as alkaloids, flavonoids, terpenes, and phenolic acids have been identified and isolated, revealing their profound effects on the human body. For example, curcumin, a polyphenol found in turmeric, has demonstrated potent anti-inflammatory and antioxidant capabilities, attracting attention as a potential treatment for conditions ranging from arthritis to neurodegenerative diseases (Patwardhan et al. 2005).

Chapter 1 contains introduction to substrate channeling in enzymes, its mechanism and biotechnological applications. It also contains literature compilation of medicinal molecules from plants, its origin and methods such as genomics and transcriptomics that enable identification of such pathways involved in production of such molecules. It also contains list of tools, databases and algorithms that aid in such a study.

Chapter 2 discusses a novel mechanism by which substrate channeling occurs in Phenyl acetate degradation pathway in bacteria. The enzyme PaaZ is used by many bacteria to degrade environmental pollutants. PaaZ is a bifunctional enzyme that catalyzes the ring opening of oxepin-CoA and converts it to 3-oxo-5,6-dehydrosuberyl-CoA. Here we report the structures of PaaZ determined by electron cryomicroscopy with and without bound ligands. The structures reveal that three domain-swapped dimers of the enzyme form a trilobed structure. A combination of small-angle X-ray scattering (SAXS), computational studies, mutagenesis and microbial growth experiments suggests that the key intermediate is transferred from one active site to the other by a mechanism of electrostatic pivoting of the CoA moiety, mediated by a set of conserved positively charged residues (Sathyanarayanan et al. 2019).

Chapter 3 describes a computational pipeline for identifying, validating, and analyzing the key components involved in the synthesis of terpenoids, a medicinally important group of phytochemicals. A bioinformatic study of this nature will have wider implication as not only a tool to understand sequence and structure–function relationships of some of the well-studied

metabolites and enzymes, to aid protein engineering for biotechnological utilization of these commercially valuable molecules (Gandhimathi et al. 2018).

Chapter 4 we describe the draft genome of *Ocimum tenuiflurum L* (*subtype Krishna Tulsi*). The paired-end and mate-pair sequence libraries were generated for the whole genome sequenced with the Illumina Hiseq 1000, resulting in an assembled genome of 374 Mb, with a genome coverage of 61 % (612 Mb estimated genome size). We have also studied transcriptomes (RNA-Seq) of two subtypes of O. tenuiflorum, Krishna and Rama Tulsi and report the relative expression of genes in both the varieties. The pathways leading to the production of medicinally-important specialized metabolites have been studied in detail, in relation to similar pathways in Arabidopsis thaliana and other plants. Expression levels of anthocyanin biosynthesis-related genes in leaf samples of Krishna Tulsi were observed to be relatively high, explaining the purple colouration of Krishna Tulsi leaves. The expression of six important genes identified from genome data were validated by performing q-RT-PCR in different tissues of five different species, which shows the high extent of urosolic acid-producing genes in young leaves of the Rama subtype. In addition, the presence of eugenol and ursolic acid, implied as potential drugs in the cure of many diseases including cancer was confirmed using mass spectrometry. The availability of the whole genome of *O.tenuiflorum* and our sequence analysis suggests that small amino acid changes at the functional sites of genes involved in metabolite synthesis pathways confer special medicinal properties to this herb (Upadhyay et al. 2015).

In Chapter 5, we describe transcriptome sequencing of an important medicinal plant, Fenugreek (*Trigonella foenum-graecum L.*). It is a self-pollinated leguminous crop belonging to the Fabaceae family. It is a multipurpose crop used as herb, spice, vegetable and forage. It is a traditional medicinal plant in India attributed with several nutritional and medicinal properties including antidiabetic and anticancer. We have performed a combined transcriptome assembly from RNA sequencing data derived from leaf, stem and root tissues. Around 209,831 transcripts were deciphered from the assembly of 92% completeness and an N50 of 1382 bases. Whilst secondary metabolites of medicinal value, such as trigonelline, diosgenin, 4-hydroxyisoleucine and quercetin, are distributed in several tissues, we report transcripts that bear sequence signatures of enzymes involved in the biosynthesis of such metabolites and are highly expressed

in leaves, stem and roots. One of the antidiabetic alkaloid, trigonelline and its biosynthesising enzyme, is highly abundant in leaves. These findings are of value to nutritional and the pharmaceutical industry (Naika et al. 2022) .

In conclusion **(Chapter 6)**, this thesis has investigated role of substrate channeling in multi-step reaction pathways. Several plant metabolites which have medicinal properties are synthesized through multi-step reaction and may employ substrate channeling as a mechanism to handle unstable intermediates. This thesis also provides a framework to combine biochemistry techniques such as substrate channeling and bioinformatics techniques like genomics, transcriptomics and evolutionary analysis of enzymes that are crucial for the bio-pharmaceutical industry.

References:-

Fabricant, D. S., and N. R. Farnsworth. 2001. "The Value of Plants Used in Traditional Medicine for Drug Discovery." *Environmental Health Perspectives* 109 (Suppl 1): 69. https://doi.org/10.1289/EHP.01109S169.

Gandhimathi, A., Nitish Sathyanarayanan, Meenakshi Iyer, Rachit Gupta, and R. Sowdhamini. 2018. "Evolutionary Analysis of a Few Protein Superfamilies in Ocimum Tenuiflorum," 37–60. https://doi.org/10.1007/978-3-319-97430-9_4.

Hand, Steven C., and Iris Hardewig. 2003. "Downregulation of Cellular Metabolism During Environmental Stress: Mechanisms and Implications." *Https://Doi.Org/10.1146/Annurev.Ph.58.030196.002543* 58 (November): 539–63. https://doi.org/10.1146/ANNUREV.PH.58.030196.002543.

Naika, Mahantesha B.N., Nitish Sathyanarayanan, Radha Sivarajan Sajeevan, Teerna Bhattacharyya, Pritha Ghosh, Meenakshi S. Iyer, Mahita Jarjapu, et al. 2022. "Exploring the Medicinally Important Secondary Metabolites Landscape through the Lens of Transcriptome Data in Fenugreek (Trigonella Foenum Graecum L.)." *Scientific Reports 2022 12:1* 12 (1): 1–13. https://doi.org/10.1038/s41598-022-17779-8.

Patwardhan, Bhushan, Dnyaneshwar Warude, P. Pushpangadan, and Narendra Bhatt. 2005. "Ayurveda and Traditional Chinese Medicine: A Comparative Overview." *Evidence-Based Complementary and Alternative Medicine* 2 (4): 465–73.

https://doi.org/10.1093/ecam/neh140.

Sathyanarayanan, Nitish, Giuseppe Cannone, Lokesh Gakhar, Nainesh Katagihallimath, Ramanathan Sowdhamini, Subramanian Ramaswamy, and Kutti R. Vinothkumar. 2019. "Molecular Basis for Metabolite Channeling in a Ring Opening Enzyme of the Phenylacetate Degradation Pathway." *Nature Communications 2019 10:1* 10 (1): 1–12. https://doi.org/10.1038/s41467-019-11931-1.

Upadhyay, Atul K, Anita R Chacko, A Gandhimathi, Pritha Ghosh, K Harini, Agnel P Joseph, Adwait G Joshi, et al. 2015. "Genome Sequencing of Herb Tulsi Ocimum Tenuiflorum Unravels Key Genes behind Its Strong Medicinal Properties." *BMC Plant Biology* 15 (1): 212. https://doi.org/10.1186/s12870-015-0562-x.

Wheeldon, Ian, Shelley D Minteer, Scott Banta, Scott Calabrese Barton, Plamen Atanassov, and Matthew Sigman. 2016. "Substrate Channelling as an Approach to Cascade Reactions." *Nature Chemistry* 8 (4): 299–309. https://doi.org/10.1038/nchem.2459.

Zhang, Y -H. Percival. 2011. "Substrate Channeling and Enzyme Complexes for Biotechnological Applications." *Biotechnology Advances* 29 (6): 715–25. https://doi.org/10.1016/j.biotechadv.2011.05.020.